METABOLIC FLUX ANALYSIS FOR ETHANOL PRODUCTION IN *Saccharomyces cerevisiae*

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Introduction. Metabolic Flux Analysis is a powerful tool for improving the understanding of cellular metabolism. It provides a confident quantitative description of the degree of involvement of different pathways in cellular functions and metabolic processes (1). In this work, we were focused on the evaluation of the metabolic flux distribution in *S. cerevisiae* and its relationship with ethanol production, for the purpose of explaining low product yields, in cellular suspensions.

Methods. Batch cultures were performed in a 5-L bioreactor using the YPD culture medium (2). Concentration of glycerol, acetic acid, ethanol and glucose were measured by HPLC; biomass was measured by spectrophotometry. The metabolic model included 33 biochemical reactions and 24 metabolites leading to 9 degrees of freedom. The underdetermined system was solved by means of an optimization problem with ethanol production as the objective function. The software CellNetAnalyzer (3) was used for this purpose.

Results. Besides the stoichiometric constraints, the experimentally determined fluxes of glycerol, acetic acid, ethanol and glucose, were used for computational flux calculations; table 1 shows the corresponding values. The resulting flux distribution provides the highest product biosynthesis yield when the carbon flux is attenuated in the oxidative pentose phosphate pathway (see figure 1), thus compromising the availability of important biomass precursors e.g. R5P and E4P. When the carbon flux heading to the oxidative PP, Ethanol yield ($\mathbf{Y_{SP}}$) is increased 56%, compared to the experimentally calculated value. Furthermore, the highest ethanol production is conditioned by the fate of the flux distribution in the pyruvate pool as a bifurcation point. The highest ethanol contraction is achieved when the carbon flux addressed to AcCoA, a TCA precursor, represents about 5 % of the total carbon flux, coming from glycolysis.

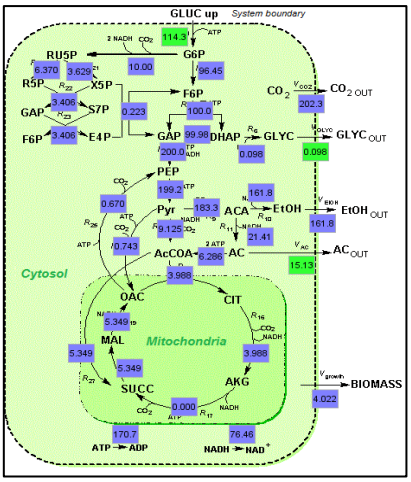

Fig.1 Metabolic flux distributions for ethanol production by *S. cerevisiae*. The values in the green boxes correspond to experimental constrains.

Table 1. Formation (or uptake) rate of extracellular metabolites, experimentally determined.

Flux	Metabolite	Rate
		mmol/ h
R_1	*Glucose*	114,394
V_{EtOH}	*Ethanol*	98,799
V_{GLYC}	*Glycerol*	0,098
V_{AC}	*Acetic acid*	15,132

Conclusions. Genetic Engineering strategies aim at improving ethanol concentration must consider the distribution of carbon fluxes without compromising availability of biomass precursors. Therefore, down regulation strategies must be implemented rather than knocking down approaches**.**

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